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AWARD NUMBER: W81XWH-14-1-0213

TITLE: Epigenetic Subtypes of Triple-Negative Breast Cancer

PRINCIPAL INVESTIGATOR: Meissner, Alexander

RECIPIENT: President and Fellows of Harvard College
Cambridge, MA 02138

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Fort Detrick, Maryland 21702-5012

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5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Alexander Meissner

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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

President and Fellows of Harvard College
1033 Massachusetts Avenue
5th Floor
Cambridge, MA 02138

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

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13. SUPPLEMENTARY NOTES

14. ABSTRACT

Triple negative breast cancer (TNBC) is the only major breast tumor subtype that lacks targeted therapy and mainly due to this has poor outcome. The majority of TNBCs have basal/stem cell-like phenotypes implying inability to respond to luminal differentiation-inducing stimuli. Using somatic cell genetics we determined that the basal phenotype is generally dominant. Based on the analysis of a limited number of TNBC cell lines we found that the epigenetic profiles of TNBCs are highly heterogeneous and subtypes defined based on chromatin modification patterns do not overlap with classification based on gene expression profiles. To further dissect the epigenetic heterogeneity of TNBCs, we have characterized the histone H3 lysine 27 acetylation (H3K27ac) profiles of a large panel (~50) TNBC cell lines and primary cultures derived from patient samples. We also included luminal cells as controls. We detected distinct clustering patterns with major luminal-basal branches. However, a subset of TNBCs, enriched for those derived from pleural effusion and distant metastases, clustered closer to luminal lines rather than to other TNBCs. This observation raises the possibility that distant metastases of TNBCs are epigenetically more luminal, which has implications for systemic therapies designed to eliminate these lesions. We have also characterized the global DNA methylation profiles of a panel of TNBC lines before and after the down regulation or pharmacologic inhibition of the KDM4C histone demethylase. Interestingly, modulating KDM4C's activity appears to lead to changes in DNA methylation patterns linking regulators of histone and DNA methylation.

15. SUBJECT TERMS

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
U Unclassified	U Unclassified	U Unclassified	Unclassified		19b. TELEPHONE NUMBER *(include area code)*

TABLE OF CONTENTS

1. **INTRODUCTION:** Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.

Triple negative breast cancer (TNBC) is a major breast tumor subtype characterized by the absence of estrogen receptor (ER) and progesterone receptor (PR) expression, and the lack of human epidermal growth factor receptor 2 (HER2) amplification. TNBC patients experience a poor clinical outcome owing to a 5-year risk of recurrence that is higher than any other subtype, notably at distant sites[1]. Cancer genome sequencing studies focusing on TNBC failed to identify novel recurrently mutated cancer-driving genes[2], obviating immediate opportunities for targeted therapeutic development. TNBC is also a heterogeneous disease[3,4], suggesting that one treatment may not suit all patients and that multiple new treatment strategies will be required.
To interrogate processes that determine luminal and basal breast cancer phenotypes and their inheritance pattern, the Polyak lab has generated somatic cell fusions and performed integrated genetic and epigenetic (DNA methylation and chromatin) profiling[5]. We found that the basal-like trait is generally dominant and it is largely defined by epigenetic repression of luminal transcription factors. Definition of super-enhancers highlighted a core program common in luminal cells but high degree of heterogeneity in basal-like breast cancers that correlates with clinical outcome. We also found that protein extracts of basal-like cells is sufficient to induce luminal-to-basal phenotypic switch implying a trigger of basal-like autoregulatory circuits. We determined that *KDM6A* might be required for luminal-basal fusions, and identified *EN1*, *TBX18*, and *TCF4* as candidate transcriptional regulators of luminal-to-basal switch. Our findings highlight the remarkable epigenetic heterogeneity of TNBCs. Based on our preliminary data we hypothesize that (1) the epigenetic profiles of TNBCs are better predictors of the functional properties and thus clinical behavior of the cells such as their ability to develop distant metastases and respond to therapeutic interventions, (2) epigenetic instability in TNBCs is associated with increased intratumor heterogeneity that drives disease progression, and (3) histone demethylases such as KDM6A are key determinants of epigenetic stability and this, at least partially, is due to their influence on DNA methylation profiles. The aim of this application is to test these hypotheses using a combination of experimental and computational approaches.

2. **KEYWORDS:** Provide a brief list of keywords (limit to 20 words).

Triple negative breast cancer, epigenetic, chromatin, DNA methylation

3. **ACCOMPLISHMENTS:** The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency Grants Officer whenever there are significant changes in the project or its direction.

What were the major goals of the project?
List the major goals of the project as stated in the approved SOW. If the application listed milestones/target dates for important activities or phases of the project, identify these dates and show actual completion dates or the percentage of completion.

The goal of this proposal is to test the hypothesis that (1) the epigenetic profiles of TNBCs are better predictors of the functional properties and thus clinical behavior of the cells such as their ability to develop distant metastases and respond to therapeutic interventions, (2) epigenetic instability in TNBCs is associated with increased intratumor heterogeneity, which drives disease progression, and (3) histone demethylases are key determinants of epigenetic stability and this, at least partially, is due to their influence on DNA methylation profiles.

Specific Aims: Aim 1 – Define epigenetic heterogeneity in TNBCs. Aim 2 – Explore the role of histone demethylases in epigenetic heterogeneity of TNBCs.

Tasks:
TASK 1: Perform RRBS analysis of TNBC cells (patient samples, xenografts from patient-derived tumors, and cell lines); Timeframe, months 1-6
TASK 2: Perform ChIP-seq analysis of TNBC cells (patient samples, xenografts from patient-derived tumors, and cell lines); Timeframe, months 1-6
TASK 3: Perform ChIP-bis-seq analysis of TNBC cells (patient samples, xenografts from patient-derived tumors, and cell lines); Timeframe, months 6-12
TASK 4: Investigate the consequences of downregulating HDMs on the phenotype of TNBCs; Timeframe, months 12-20
TASK 5: To complete experiments, analyze data and submit it for publication; Timeframe, months 20-24

What was accomplished under these goals?
For this reporting period describe: 1) major activities; 2) specific objectives; 3) significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative); and/or 4) other achievements. Include a discussion of stated goals not met. Description shall include pertinent data and graphs in sufficient detail to explain any significant results achieved. A succinct description of the methodology used shall be provided. As the project progresses to completion, the emphasis in reporting in this section should shift from reporting activities to reporting accomplishments.

1) Major activities:
Polyak lab:
We have generated primary cultures from TNBCs. We have generated and characterized derivatives of TNBCs expressing TET-inducible shRNAs for KDM4C histone demethylase (HDM). We have performed H3K27ac ChIP-seq profiling of a large panel of TNBC cell lines and primary cultures of patient-derived TNBCs. We have prepared genomic DNA from TNBCs for DNA methylation studies by the Meissner lab.

Meissner lab:
We have obtained TNBC cell line DNA from the Polyak lab and following standard QC assessment processed them into reduced representation bisulfite sequencing libraries. All libraries were sequenced on the Illumina HiSeq2500 and processed using our custom QC and analysis pipeline. On average we obtained 16,843,250 aligned reads and captured on average more than 3 million CpGs with a mean coverage of 10 reads. Preliminary analysis using hierarchical clustering and detection of differentially methylated regions (DMRs) was completed. Additional in depth analysis and pairwise sample comparisons are ongoing.

2) Specific objectives:
Polyak lab:
Characterize the histone modification profiles of TNBC cell lines, patient samples and cell culture/xenograft derived from these. Prepare genomic DNA from the same samples for DNA methylation profiling.

Meissner lab:
Perform DNA methylation profiling of TNBCs and perform integrative bioinformatics analysis.

3) Significant results or key outcomes:

Progress towards achieving Specific Aim 1
(Define epigenetic heterogeneity in TNBCs):

Polyak lab:
In the past 12 months, we have assembled a large collection of TNBC cell lines (~50 cell lines) reflecting diverse subtypes of TNBCs (e.g., basal, mesenchymal, luminal AR+). We have also developed several novel patient-derived xenograft (PDX) models of TNBCs and we are currently expanding these to be used for epigenetic profiling as well as for preclinical testing of inhibitors of epigenetic regulators. We have tested the sensitivity of these TNBC cell lines to various epigenetic agents such as bromodomain, EZH2, KDM6, KDM4C, KDM5B, and DNMT inhibitors. We have also generated RNA-seq and H3K27ac ChIP-seq from all the cell lines. The reason we picked H3K27ac profiles is because these are marking enhancers and superenhancers, which play key roles in defining cellular identity, thus, we feel may provide useful information on epigenetic heterogeneity of TNBCs. We have analyzed the majority of these libraries and performed preliminary clustering analyses of the samples (**Figure 1**).

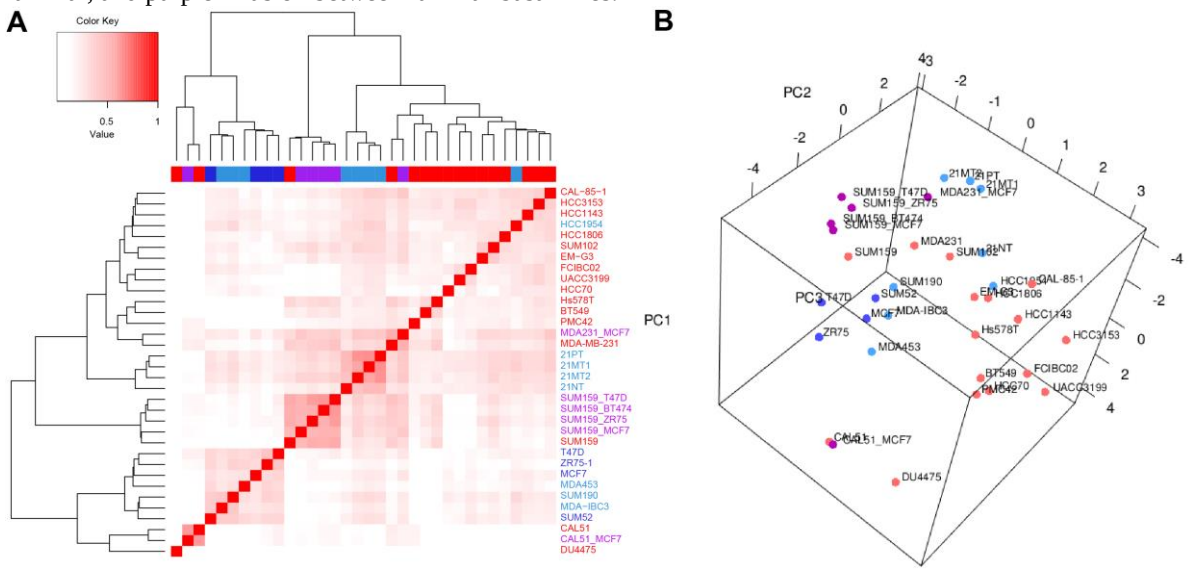


Figure 1. Heatmap (A) and 3D PCA plot (B) depicting the relatedness of breast cancer cell lines based on H3K27ac profiles. Colors reflect cell line subtypes: red-TNBC, light blue – HER2+ luminal, dark blue – ER+ luminal, and purple – fusion between luminal-basal lines.

Meissner lab:
We have completed the data generation and initial analysis for the first set of TNBC samples. The results suggest high variability for DNA methylation among cell lines, even within the same tumor subtype. For example, while in general TNBCs are more hypomethylated than luminal breast cancers, some TNBCs are more hypermethylated than some luminal cell lines. Interestingly, this feature appears to be associated with responsiveness to luminal differentiation inducing transcription factors implying a link between DNA methylation and differentiation.

Progress made towards achievement of Specific Aim 2
(Explore the role of histone demethylases in epigenetic heterogeneity of TNBCs):

Polyak lab:
We have generated derivatives of several TNBC (e.g., SUM149) and control luminal (e.g., T47D) breast cancer cell lines that stable express TET-inducible shRNAs targeting KDM4C/JMJD2C

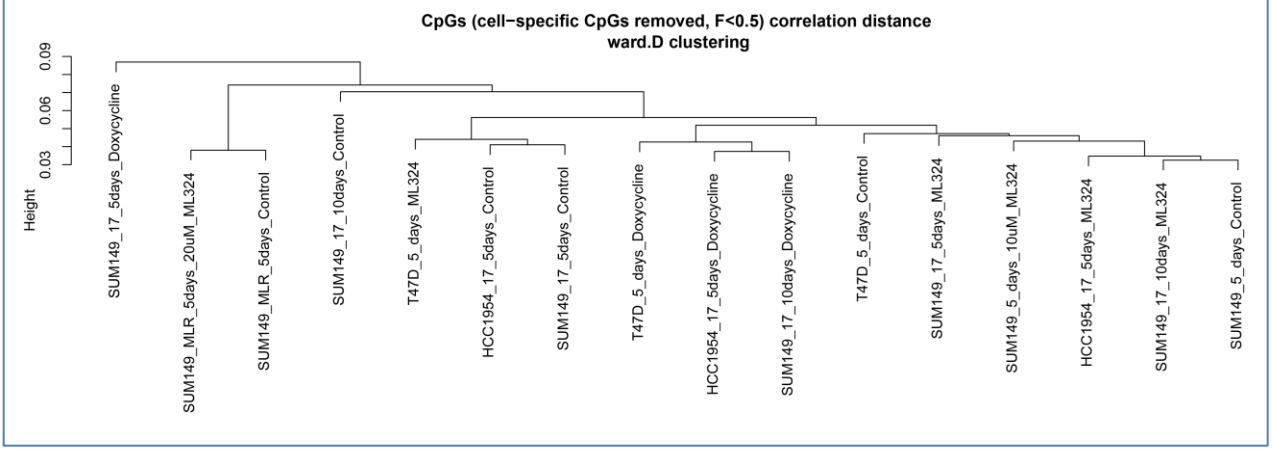
H3K9/H3K36 histone demethylase. We have characterized the gene expression and histone modification profiles of these cells before and after long-term downregulation of JMJD2C. We have also tested the effects of pharmacologic inhibition of JMJD2C using ML324 and derived TNBC cell lines that are resistant to the growth inhibitory effects of ML324. We have RNA-seq, ChIP-seq (for JMJD2C, H3K9, and H3K36), and DNA methylations data from all the cell lines and conditions and currently performing integrated analyses.

Meissner lab:

To determine the effect of pharmacologic inhibitor (ML324) or JMJD2C shRNA on the DNA methylation landscape in the TNBCs, we performed pairwise comparisons (e.g. HCC_control vs HCC_ Dox, HCC_control vs HCC_ML324 etc) and used Fisher's exact test with a p-value cutoff of $p<0.01$ and absolute methylation change of >10% to identify differentially-methylated CpGs (DMCs). Overlapping DMCs in each sample are counted by intersecting the two tests for each cell (e.g. HCC_control vs HCC_ Dox and HCC_control vs HCC_ML324), and finally the overlap of all DMCs in all tests. As an example, the number of DMCs in the HCC are as follows (similar range is found for the SUM lines): 35,796 HCC_control vs HCC_Dox, 40,020 HCC_control vs HCC_ML324 and 9,610 All HCC. Next an ANOVA, which measures the ratio (F) of among-sample-variance to within-sample-variance, was used for clustering at three F values: 2, 1, and 0.5, representing increasing levels of stringency to filter out CpGs that show differences between cell types.

There are 1,087,764 total CpGs with coverage of 5x or more in 80% of the samples. For the F<2

Figure 2. Clustering of samples based on DNA methylation profiles.



cutoff (the ratio of among-sample-variance could be as much as 2 times greater than the within-sample variance) there were 250,097 CpGs. For the $F < 1$ cutoff (the ratio of among-sample-variance must be less than within-sample variance) there were 149,132 CpGs. For the $F < 0.5$ cutoff (less among-sample variance and more within-sample variance) there were 83,361 CpGs (of these, 24,747 are covered at 5x coverage in all samples). Despite the preliminary nature of the analysis we do seem to detect significant effects of the treatments on the DNA methylation landscape. A preliminary cluster analysis of the samples analyzed is depicted in **Figure 2.** These results will be further validated using additional replicates and analyses.

Key research accomplishments

A major focus of this project is to test the hypothesis that the epigenetic profiles of TNBCs are heterogeneous and that classification based on epigenetic profiles is distinct from that one gene expression and it is clinically more useful. In this reporting period we explored the H3K27ac profiles of ~30 TNBC cell lines and we found distinct subset that did not correlate with expression-based classification.

Key accomplishments:

(1) Generating preliminary evidence for the first time that the H3K27ac and super-enhancer profiles of TNBCs is heterogeneous and classifies samples into different subsets than expression data.
(2) We found that downregulation or pharmacologic inhibition of the JMJD2C histone demethylase alters global DNA methylation patterns implying a link between JMJD2C activity and DNMTs.

Conclusion

In the first year of this project we have established consistent and high-throughput ChIP-seq protocols and data analyses pipelines. We are currently expanding our analyses to clinical samples and additional treatment groups.

4) Other achievements:
None

What opportunities for training and professional development has the project provided?
If the project was not intended to provide training and professional development opportunities or there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe opportunities for training and professional development provided to anyone who worked on the project or anyone who was involved in the activities supported by the project. "Training" activities are those in which individuals with advanced professional skills and experience assist others in attaining greater proficiency. Training activities may include, for example, courses or one-on-one work with a mentor. "Professional development" activities result in increased knowledge or skill in one's area of expertise and may include workshops, conferences, seminars, study groups, and individual study. Include participation in conferences, workshops, and seminars not listed under major activities.

Guillermo Peluffo (Polyak lab) gave oral presentations at the DFCI-MIT annual PPG retreat in Colraine, MA, DFCI CFCE (Center for Functional Epigenetics) annual retreat in August, and at the Broad Institute epigenetic seminars series in September

How were the results disseminated to communities of interest?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the results were disseminated to communities of interest. Include any outreach activities that were undertaken to reach members of communities who are not usually aware of these project activities, for the purpose of enhancing public understanding and increasing interest in learning and careers in science, technology, and the humanities.

Guillermo Peluffo (Polyak lab) gave oral presentations at the DFCI-MIT annual PPG retreat in Colraine, MA, DFCI CFCE (Center for Functional Epigenetics) annual retreat in August, and at the Broad Institute epigenetic seminars series in September

What do you plan to do during the next reporting period to accomplish the goals?
If this is the final report, state "Nothing to Report."

Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.

We will characterize the chromatin and DNA methylation profiles of primary patient-derived TNBCs to improve our understanding of epigenetic heterogeneity of TNBCs. We will also develop derivatives of TNBC cell lines with TET-inducible shRNAs targeting HDMs and characterize DNA methylation and histone modification changes following the downregulation or pharmacologic inhibition of HDMs.

4. **IMPACT:** Describe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:

What was the impact on the development of the principal discipline(s) of the project?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how findings, results, techniques that were developed or extended, or other products from the project made an impact or are likely to make an impact on the base of knowledge, theory, and research in the principal disciplinary field(s) of the project. Summarize using language that an intelligent lay audience can understand (Scientific American style).

We (Polyak lab) have collected clinical samples, developed PDX and primary cell culture models of TNBC, developed derivatives of TNBC cell lines with decreased JMJD2C activity due to shRNA-mediated downregulation or pharmacologic inhibition. We (Meissner lab) have performed global DNA methylation profiling of TNBCs following JMJD2C inhibition and developed data analysis tools for integrating DNA methylation, ChIP-seq, and RNA-seq profiles.

What was the impact on other disciplines?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the findings, results, or techniques that were developed or improved, or other products from the project made an impact or are likely to make an impact on other disciplines.

Nothing to report

What was the impact on technology transfer?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe ways in which the project made an impact, or is likely to make an impact, on commercial technology or public use, including:

- *transfer of results to entities in government or industry;*
- *instances where the research has led to the initiation of a start-up company; or*
- *adoption of new practices.*

Nothing to report

What was the impact on society beyond science and technology?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how results from the project made an impact, or are likely to make an impact, beyond the bounds of science, engineering, and the academic world on areas such as:

- *improving public knowledge, attitudes, skills, and abilities;*
- *changing behavior, practices, decision making, policies (including regulatory policies), or social actions; or*
- *improving social, economic, civic, or environmental conditions.*

Nothing to report

5. **CHANGES/PROBLEMS:** The Project Director/Principal Investigator (PD/PI) is reminded that the recipient organization is required to obtain prior written approval from the awarding agency Grants Officer whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, "Nothing to Report," if applicable:

Changes in approach and reasons for change
Describe any changes in approach during the reporting period and reasons for these changes. Remember that significant changes in objectives and scope require prior approval of the agency.

Nothing to report

Actual or anticipated problems or delays and actions or plans to resolve them
Describe problems or delays encountered during the reporting period and actions or plans to resolve them.

Nothing to report

Changes that had a significant impact on expenditures
Describe changes during the reporting period that may have had a significant impact on expenditures, for example, delays in hiring staff or favorable developments that enable meeting objectives at less cost than anticipated.
Nothing to report

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents
Describe significant deviations, unexpected outcomes, or changes in approved protocols for the use or care of human subjects, vertebrate animals, biohazards, and/or select agents during the reporting period. If required, were these changes approved by the applicable institution committee (or equivalent) and reported to the agency? Also specify the applicable Institutional Review Board/Institutional Animal Care and Use Committee approval dates.

Significant changes in use or care of human subjects

Nothing to report

Significant changes in use or care of vertebrate animals.

Polyak Lab Animal Study reviewed and approved by Institutional Animal Care and Use Committee for period 10/6/15-10/6/16.

Significant changes in use of biohazards and/or select agents

Nothing to report

6. **PRODUCTS:** List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."

- **Publications, conference papers, and presentations**
  Report only the major publication(s) resulting from the work under this award.

  Journal publications. *List peer-reviewed articles or papers appearing in scientific, technical, or professional journals. Identify for each publication: Author(s); title; journal; volume: year; page numbers; status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

  Su Y, Subedee A, Bloushtain-Qimron N, Savova V, Krzystanek M, Li L, Marusyk A, Tabassum DP, Zak A, Flacker MJ, Li M, Lin JJ, Sukumar S, Suzuki H, Henry Long H, Szallasi Z, Alexander Gimelbrant A, Maruyama R, **Polyak K**. Somatic cell fusions reveal extensive heterogeneity in basal-like breast cancer. Cell Reports 2015; 11:1549-1563.

  Books or other non-periodical, one-time publications. *Report any book, monograph, dissertation, abstract, or the like published as or in a separate publication, rather than a periodical or series. Include any significant publication in the proceedings of a one-time conference or in the report of a one-time study, commission, or the like. Identify for each one-time publication: Author(s); title; editor; title of collection, if applicable; bibliographic information; year; type of publication (e.g., book, thesis or dissertation); status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

Nothing to report

  Other publications, conference papers, and presentations. *Identify any other publications, conference papers and/or presentations not reported above. Specify the status of the publication as noted above. List presentations made during the last year (international, national, local societies, military meetings, etc.). Use an asterisk (*) if presentation produced a manuscript.*

Nothing to report

- **Website(s) or other Internet site(s)**
  List the URL for any Internet site(s) that disseminates the results of the research activities. A short description of each site should be provided. It is not necessary to include the publications already specified above in this section.

  Nothing to report

- **Technologies or techniques**
  Identify technologies or techniques that resulted from the research activities. In addition to a description of the technologies or techniques, describe how they will be shared.

Nothing to report

- **Inventions, patent applications, and/or licenses**
  Identify inventions, patent applications with date, and/or licenses that have resulted from the research. State whether an application is provisional or non-provisional and indicate the

application number. Submission of this information as part of an interim research performance progress report is not a substitute for any other invention reporting required under the terms and conditions of an award.

Nothing to report

- **Other Products**

  Identify any other reportable outcomes that were developed under this project. Reportable outcomes are defined as a research result that is or relates to a product, scientific advance, or research tool that makes a meaningful contribution toward the understanding, prevention, diagnosis, prognosis, treatment, and/or rehabilitation of a disease, injury or condition, or to improve the quality of life. Examples include:
  - *data or databases;*
  - *biospecimen collections;*
  - *audio or video products;*
  - *software;*
  - *models;*
  - *educational aids or curricula;*
  - *instruments or equipment;*
  - *research material (e.g., Germplasm; cell lines, DNA probes, animal models);*
  - *clinical interventions;*
  - *new business creation; and*
  - *other.*

  Nothing to Report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort). If information is unchanged from a previous submission, provide the name only and indicate "no change."

Name: Kornelia Polyak
Project Role: PI
Researcher Identifier (e.g. ORCID ID): 0000-0002-5964-0382
Nearest person month worked: 2

Contribution to Project: Dr. Polyak has supervised the project in her lab and coordinated collaboration with the Lindquist lab.
Funding Support: Please see previously provided other support and changes noted below.

Name: Guillermo Peluffo (Polyak lab)
Project Role: research fellow
Researcher Identifier (e.g. ORCID ID): 0000-0003-0954-7996
Nearest person month worked: 9
Contribution to Project: Dr. Peluffo generated and characterized derivatives of TNBC cell lines expressing TET-inducible shRNAs to KDM4C. He also generated H3K27ac ChIP-seq libraries from a large panel of TNBCs.

Funding Support: Additional funds from Dr. Polyak's grants from Novartis.

Name: Alexander Meissner
Project Role: Partnering PI
Researcher Identifier (e.g. ORCID ID): 0000-0001-1846-7469
Nearest person month worked: 1

Contribution to Project: Dr. Meissner has supervised the project in his lab and coordinated collaboration with the Polyak lab.
Funding Support: Please see previously provided other support and changes noted below.

Name: Kendell Clement (Meissner lab)
Project Role: Graduate student
Researcher Identifier (e.g. ORCID ID): 0000-0003-3808-0811
Nearest person month worked: 4
Contribution to Project: Mr. Clement analyzed RRBS profiles of TNBCs.
Funding Support:

Name: Michael Ziller (Meissner lab)
Project Role: Postdoctoral Researcher
Researcher Identifier (e.g. ORCID ID): 0000-0003-0781-7980
Nearest person month worked: 9
Contribution to Project: Dr. Ziller maintains the essential computational pipelines and infrastructure used to process the RRBS profiles of TNBCs. He is involved in the data analysis and interpretation.
Funding Support: Additional funds from Dr. Meissner's grants.

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

If the active support has changed for the PD/PI(s) or senior/key personnel, then describe what the change has been. Changes may occur, for example, if a previously active grant has closed and/or if a previously pending grant is now active. Annotate this information so it is clear what has changed from the previous submission. Submission of other support information is not necessary for pending changes or for changes in the level of effort for active support reported previously. The awarding agency may require prior written approval if a change in active other support significantly impacts the effort on the project that is the subject of the project report.

Changes in Kornelia Polyak's other support:

Grants ended (September 2014 – present):
Breast Cancer Alliance - Exceptional Project Grant (Polyak)
Microenvironment-induced metabolic alterations and therapeutic resistance in breast cancer
01/01/2014-12/31/2014

Novartis-DFCI Drug Discovery Program (Polyak)
JMJD2C histone demethylase as therapeutic target in breast cancer
01/01/2013 – 12/31/2014

Susan G. Komen Foundation Promise grant (Garber-Polyak)

KG101097 Preclinical & Brief Exposure Early Clinical Evaluation of an Oral PARP Inhibitor

5/25/10-5/24/15

New grants awarded: **(September 2014 – present)**

U01 CA195469 (Polyak/Michor/Spellman/Gray) 06/01/15 – 05/31/20 0.60 Calendar Months
NIH/NCI $503,808 (5% Effort)
Role: Principal Investigator

Intratumor heterogeneity underlying treatment resistance in HER2+ breast tumors

Specific Aims: 1) Develop a multi-scale model of primary and metastatic breast tumors; Aim 2) Parameterize the multi-scale mathematical model based on data from mouse xenograft models; Aim 3) Use the multi-scale model to predict disease kinetics and optimum prevention and treatment strategies, and validate these strategies in mouse xenograft models.

POC: Rebecca Brightful-Grants Management Specialist; Email: brightfr@mail.nih.gov; Phone: 301-631-3011

U54 CA193461 (Michor) 05/19/15 – 04/30/20 0.60 Calendar Months
NIH/NCI $210,600 (5% Effort)
Role: Project 3 Principal Investigator

Evolution and Treatment Response of Brain, Breast, and Hematologic Malignancies – Project 3

Single Cell Measures of Intratumor Diversity for Optimal Breast Cancer Therapy

The Dana-Farber Cancer Institute-Physical Sciences-Oncology Center (DFCI-PSOC) will bring together a trans-disciplinary research team to advance our understanding of the physical principles that govern the response of tumor cell populations to treatment and the emergence of resistance.

Specific Aims – Project 3: 1) Perform single cell analyses of breast tumor samples; (2) Characterize therapeutic responses in xenograft models of breast cancer; and 3) Predict optimal therapeutic strategies to prevent metastatic outgrowth and treatment resistance and validate these strategies in xenograft models.

Breast Cancer Research Foundation (Polyak) 10/02/08 – 09/30/15 .84 Calendar Months
Innovative Research Grant $208,333 (7% Effort)
Role: Principal Investigator

Molecular basis of breast tumor heterogeneity and its clinical consequences

The major goal of this grant is to determine the mechanisms underlying intra-tumor heterogeneity and their clinical relevance.

POC: Deputy Director: Margaret Mastrianni Email: mailto:pegmast@bcrfcure.org Phone: (646) 497-2600

(Polyak) 01/01/15 – 12/31/17 0.06 Calendar Months
DFCI-NOVARTIS Drug Discovery Program $123,524 (.5% Effort)
Role: Principal Investigator

Integrated analysis of heterogeneity in and drivers of metastatic cancers

Specific Aims: 1) Determine the contribution of genetic heterogeneity to metastasis in breast cancer; 2) Develop strategies to assess genomic heterogeneity in human tumors; and 3) Develop methods to generate models of metastatic tumors.

POC: Program Administrator Sylvia C. Lin Email**:** Sylvia_Lin@dfci.harvard.edu Phone**:** (617) 632-5599

(Polyak/Brown/Roberts/Shivdasani/Stegmaier) 01/01/15 – 12/31/17 0.06 Calendar Months

DFCI-NOVARTIS Drug Discovery Program $350,000 (.5% Effort)
Role: Principal Investigator
Epigenetic dependencies in human cancer
Specific Aims: 1) Identify changes in epigenetic dependencies following pharmacologic perturbations; 2) Identify dependencies of cancer cells resistant to epigenetic modulators; and 3) Perform a genome-wide CRISPR screen to investigate mechanisms of resistance to epigenetic therapies.
POC: Program Administrator Sylvia C. Lin Email**:** Sylvia_Lin@dfci.harvard.edu Phone**:** (617) 632-5599

Ludwig Center at Harvard (Brugge, Demetri) 03/01/15-02/29/16 0.06 Calendar Months
Ludwig Center $150,000 (.5% Effort)
Role: Principal Investigator
Epigenetic heterogeneity in breast cancer
POC: Jane Staunton, PhD-Director of Scientific Administration and Education; Email: jane_staunton@hms.harvard.edu; Phone: 617-432-5920

R35CA19762 (Polyak) 08/01/15 – 07/31/22 6.0 Calendar Months
NIH/NCI $433,067 (50% Effort)
Role: Principal Investigator
Targeting intratumor heterogeneity in breast cancer
Specific Aims: The proposal aims at delineating tumor evolutionary paths in experimental and in clinical breast cancer using multidisciplinary approaches requiring (1) the development and application of technologies that allow for the in depths characterization of human tumors as a whole at the single cell level and in intact tissue samples, (2) the development and utilization of experimental models that more faithfully reproduce the heterogeneity of human disease, and (3) interdisciplinary approaches that incorporate molecular, mathematical, ecological, and evolutionary principles and methodologies. T
POC: Rebecca Brightful-Grants Management Specialist; Email: brightfr@mail.nih.gov; Phone: 301-631-3011

Changes in Alexander Meissner's other support:

Grants ended (September 2014 – present):

A18567 (Meissner) 09/01/12-01/01/15 0.1CM
Life Technologies Inc.
Scorecard 2.0 $114,588
Aim: The goal of this project is to demonstrate that the ScoreCard approach reported with array methods can be adapted to current TaqMan qPCR-based and Next Gen Ion Torrent analysis platforms from Life Technologies. The specific goals are to demonstrate the integration of

simplified work flow, build prototypes with extended and reduced transcriptome content and create analysis tools to reduce the time or cost or labor required for pluripotent and functional characterization of iPSC clones.

No Award No. (Meissner/Nachman) 06/1/2011-05/31/2015 0CM
Human Frontier Science Program $113,636
Studying dynamics of cell state transitions during reprogramming using a live imaging approach Goals/Aims: We aim to advance our understanding of the dynamics and mechanisms of cell state transitions during mammalian cell reprogramming using a combined high-resolution live cell imaging and probabilistic modeling approach.

<u>New grants awarded</u>: (September 2014 – present)

3R01DA036898-03 (Meissner) 08/01/2015-07/31/2016 0CM
NIH Administrative Supplement $88,757
Generation and characterization of tools for target-specific e novo DNA methylation
Goals/Aims: This project goal is to overcome the inability to manipulate DNA methylation by designing an innovative approach for targeted manipulation of DNA methylation, in a unique cellular system that also enables accurate measurements of such performance.

1R01HD078679 (McCarrey/Meissner) 09/01/2014-08/31/2019
0.6CM NIH
$138,824
Epimutations in Offspring Produced by Assisted Reproductive
Goals/Aims: The goals of this project are to determine the genome-wide extent and functional impact of epimutations induced in offspring of different ages produced by ICSI; to determine the timing of induction and correction of epimutations in ART (assisted reproductive technologies) offspring; and to determine which aspects of the ART process lead to the induction of epimutations in offspring.

U01HG007610 (Kellis/Meissner) **6/2/14-3/31/16** 0.6CM
NIH $391,022
Epigenomic variation atlas across human tissues and individuals in GTEx

What other organizations were involved as partners?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe partner organizations – academic institutions, other nonprofits, industrial or commercial firms, state or local governments, schools or school systems, or other organizations (foreign or domestic) – that were involved with the project. Partner organizations may have provided financial or in-kind support, supplied facilities or equipment, collaborated in the research, exchanged personnel, or otherwise contributed.
Provide the following information for each partnership:
<u>Organization Name:</u>

Location of Organization: (if foreign location list country)
Partner's contribution to the project (identify one or more)

- *Financial support;*
- *In-kind support (e.g., partner makes software, computers, equipment, etc., available to project staff);*
- *Facilities (e.g., project staff use the partner's facilities for project activities);*
- *Collaboration (e.g., partner's staff work with project staff on the project);*
- *Personnel exchanges (e.g., project staff and/or partner's staff use each other's facilities, work at each other's site); and*
- *Other.*

Nothing to report

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: For collaborative awards, independent reports are required from BOTH the Initiating PI and the Collaborating/Partnering PI. A duplicative report is acceptable; however, tasks shall be clearly marked with the responsible PI and research site. A report shall be submitted to **https://ers.amedd.army.mil** for each unique award.

QUAD CHARTS: If applicable, the Quad Chart (available on https://www.usamraa.army.mil) should be updated and submitted with attachments.

9. APPENDICES:

Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc.

References

1 Vaz-Luis, I. *et al.* Outcomes by tumor subtype and treatment pattern in women with small, node-negative breast cancer: a multi-institutional study. *J Clin Oncol* **32**, 2142-2150, (2014).

2 Shah, S. P. *et al.* The clonal and mutational evolution spectrum of primary triple-negative breast cancers. *Nature*, (2012).

3 Lehmann, B. D. *et al.* Identification of human triple-negative breast cancer subtypes and preclinical models for selection of targeted therapies. *J Clin Invest* **121**, (2011).

4 Metzger-Filho, O. *et al.* Dissecting the heterogeneity of triple-negative breast cancer. *J Clin Oncol* **30**, 1879-1887, (2012).

5 Su, Y. *et al.* Somatic Cell Fusions Reveal Extensive Heterogeneity in Basal-like Breast Cancer. *Cell reports* **11**, 1549-1563, (2015).